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REMARKS

Claims 1-41 were present in the application as filed. In response to a restriction requirement mailed April 17, 2003, Applicant provisionally elected claims 1-11, 17-18, 29, 33, 34, 38 and 39 with traverse with respect to claims 12-16, 19-28, 30-32, 35-37 and 40-41. Claims 1-41 were, therefore, pending in the application, with claims 30-32 withdrawn from consideration. Claims 30-32 are canceled above. Claims 1-29 and 33-41, therefore, are pending in the application. Reconsideration of the application in view of the following remarks is respectfully requested.

Applicant acknowledges the allowability of claims 17-20 and 23-28.

Rejections under 35 U.S.C.§102

Claims 1-16, 21, 22, 29 and 33-39 are rejected under 35 U.S.C. §102(b) as being anticipated by Sikorski et al. (*Genetics* 122: 19-27, 1989) and in the alternative by Palmeros et al. (*Gene* 247: 255-264, 2000).

Anticipation under 35 U.S.C. §102

"A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." Verdegaal Bros., Inc. v. Union Oil Co. of California, 814 F.2d628, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987)

Anticipation under 35 U.S.C.§102, therefore, requires the presence in a single prior art disclosure of each and every element of a claimed invention. Applicant respectfully submits, that while Sikorski et al. teach the construction of various plasmids, the reference fails to teach a

plasmid comprising (a) at least one spacer segment comprising a nucleic acid sequence that is restriction site-free; and (b) at least two polylinker regions containing a plurality of unique restriction sites as required by Applicant's claims and, therefore, cannot anticipate Applicant's claimed invention.

Independent claim 1 requires that a plasmid in accordance with the present invention comprise (a) at least one spacer segment comprising a nucleic acid sequence *that is restriction site-free*; and (b) *at least two* polylinker regions containing a plurality of *unique* restriction sites distributed so that digestion of the plasmid with any two restriction endonucleases whose sites are represented on said plasmid results in *two* fragments, said fragments being sufficiently different in size from the intact plasmid so as to be readily distinguishable from said plasmid. The fact that digestion of the plasmid results in only two fragments is significant in that it reinforces the concept that each restriction site represented on the plasmid appears only once. Referring to Figure 1 of the instant application, it is also clear that for any one plasmid of the present invention, each restriction site in the plasmid is unique, that is, it appears only once in the plasmid.

Sikorski et al. teach a series of yeast shuttle vectors derived from pBLUESCRIPT that possess all the attributes of pBLUESCRIPT and several yeast-specific features as well (abstract). The plasmids of Sikorski et al., however, differ from the claimed plasmid in a number of aspects. For example, the plasmids of Sikorski et al. contain only one polylinker as that term is known and used in the art. Secondly, the polylinker of the plasmid taught by Sikorski et al contains some restriction sites that are not unique.

The Office Action states that "each of the plasmids (taught by Sikorski) comprises at least two short segments of nucleic acids comprising more than one restriction endonuclease site (e.g., a polylinker as set forth in the claims)..." The plasmid pRS303 which appears in the

uppermost left portion of Figure 2 on page 22 of Sikorski et al. is characterized by the Office Action as having two polylinkers, the first comprising the region of the plasmid between bases 2073 and 2175 and a second polylinker at 1122-1183.

As a preliminary matter, the characterization of a polylinker as merely a short segment of nucleic acid comprising more than one restriction endonuclease is inconsistent with the term as it is known in the art and how it is defined in the instant specification (paragraph [0030]); a polylinker as defined therein is a short segment of nucleic acid having *several unique* restriction endonuclease recognition sites. Other sources define polylinkers as "synthetic oligonucleotides composed of one copy of several different restriction sites" (from *Molecular Cell Biology*, Lodish et al. Editors, W.H. Freeman and Co., publishers, 2000; see also definition of polylinker from the Glossary of *An Introduction to Genetic Analysis,* Griffiths et al. Editors, W.H. Freeman and Co., publishers, 2000; copies of both are included herewith for the Examiner's convenience).

Thus, the short segment of nucleic acid containing the two restriction endonuclease recognition sites, KpnI and PstI, at positions 1122 and 1183 respectively, which the Office Action characterizes as a polylinker is clearly not a polylinker. Rather, the enumerated sites are naturally occurring sites within the yeast *HIS3* gene that is a component of the pRS303 plasmid. Furthermore, since both sites are also found in the polylinker they are not unique to either the first polylinker or the plasmid. The polylinker contains additional sites that are also not unique; sites that are no longer unique have been underlined (see figure legend for Figure 2).

Palmeros et al. teach a plasmid, pLox1 into which a removable antibiotic resistance marker cassette can be introduced. The plasmid of Palmeros et al. has two polylinker regions or multiple cloning sites, namely L-MCS and R-MCS. These polylinkers, however, contain several restrictions sites in common, as indicated by an asterisk (see figure legend for Figure 1 on page 258.) Thus, the polylinkers fail to meet the criterion of claim 1 that the at least two polylinker

regions contain a plurality of unique restriction sites. Palmeros et al. therefore, fails to teach Applicant's claimed invention.

Since neither Sikorski et al. nor Palmeros et al. teaches or fairly suggests a plasmid having all the features recited in claim 1 of the present invention, that is, containing at least two polylinker regions containing a plurality of unique restriction sites, neither reference can anticipate the claimed invention. Withdrawal of the rejection is respectfully requested.

For the foregoing reasons, the claims are believed in condition for allowance and such action is respectfully requested. The dependent claims are believed allowable for the same reasons as the independent claims from which they ultimately depend, as well as for their additional limitations. Should the Examiner require clarification of any of the above, the Examiner is invited to contact Applicant's undersigned attorney at the telephone number listed below.

Respectfully submitted,

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polyacrylamide A material used to make electrophoretic gels for the separation of mixtures of macromolecules.

poly (A) tail A string of adenine nucleotides added to mRNA after transcription.

polycistronic mRNA An mRNA that encodes more than one protein.

polydactyly More than five fingers or toes or both. Inherited as an autosomal dominant phenotype.

polygenes *See* multiple-factor hypothesis.

polylinker A vector DNA sequence containing multiple unique restriction-enzyme-cut sites, convenient for inserting foreign DNA.

polymerase chain reaction (PCR) A method for amplifying specific DNA segments that exploits certain features of DNA replication.

polymorphism The occurrence in a population (or among populations) of several phenotypic forms associated with alleles of one gene or homologs of one chromosome.

polypeptide A chain of linked amino acids; a protein.

polyploid A cell having three or more chromosome sets or an organism composed of such cells.

polysaccharide A biological polymer composed of sugar subunits—for example, starch or cellulose.

polytene chromosome A giant chromosome produced by an endomitotic process in which the multiple DNA sets remain bound in a haploid number of chromosomes.

position effect Used to describe a situation in which the phenotypic influence of a gene is altered by changes in the position of the gene within the genome.

position-effect variegation Variegation caused by the inactivation of a gene in some cells through its abnormal juxtaposition with heterochromatin.

positional information The process by which chemical cues that establish cell fate along a geographic axis are established in a developing embryo or tissue primordium.

positive assortative mating A situation in which like phenotypes mate more commonly than expected by chance.

positive control Regulation mediated by a protein that is required for the activation of a transcription unit.

- • The most commonly used cloning vectors are *E. coli* plasmids, small circular DNA molecules that include three functional regions: (1) an origin of replication, (2) a drug-resistance gene, and (3) a region where DNA can be inserted without interfering with plasmid replication or expression of the drug-resistance gene.
- • Two enzymes are used to produce recombinant plasmids. Restriction enzymes cut DNA at specific 4- to 8-bp sequences, often leaving self-complementary single-stranded tails (sticky ends). These enzymes are used to cut long DNA molecules into multiple restriction fragments and to cut a plasmid vector at a single site. If a restriction fragment and cut plasmid vector with complementary ends are mixed under the proper conditions, DNA ligase will form phosphodiester bonds between the restriction fragment and vector DNA (see Figure 7-7).
- • When recombinant plasmids are incubated with *E. coli* cells first treated with a high concentration of divalent cations, a very small fraction of the cells take up a single recombinant plasmid. These transformed cells, which carry the plasmid drug-resistance gene, can be selected by plating on nutrient agar containing the antibiotic (see Figure 7-3). All the cells in each colony that grows on this medium contain identical plasmids descended from the single plasmid that entered the founder cell of the colony. Isolated colonies thus represent clones of the different restriction fragments originally inserted into the plasmid vector.
- • Polylinkers are synthetic oligonucleotides composed of one copy of several different restriction sites. Plasmid vectors that contain a polylinker will be cut only once by multiple restriction enzymes, each acting at its own site. Inclusion of a polylinker in a plasmid vector thus permits cloning of restriction fragments generated by cleavage of DNA with multiple different restriction enzymes.
- • Single-stranded DNA containing up to 100 nucleotides of any desired sequence can be chemically synthesized using automated instruments. Synthetic dsDNAs are produced by synthesizing complementary ssDNAs and then hybridizing them.